IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Mar Tormo Ana M. Tari Gabriel Lopez-Berestein	§	
Serial No. 08/726,211	§	Group Art Unit: 1805
Filed: October 4, 1996	§	Examiner: R. Schwartzman
For: INHIBITION OF BCL-2 PROTEIN EXPRESSION BY LIPOSOMAL ANTISENSE OLIGODEOXYNUCLEOTIDES	§	Atty. Dkt.: UTXC:504/COD

(Considered KAL 04-29-01)

DECLARATION OF ANA M. TARI AND GABRIEL LOPEZ-BERESTEIN UNDER 37 C.F.R. §1.132

WE, DR. ANA M. TARI AND DR. GABRIEL LOPEZ-BERESTEIN DECLARE AS FOLLOWS:

1. We, along with Dr. Mar Tormo, are co-inventors of the subject matter of the captioned patent application USSN 08/726,211.

2. It is our understanding that the Examiner in charge of the captioned application has rejected claims 10-20 on the grounds that the *in vitro* results in the specification are not sufficient to predict *in vivo* success in the practice of the invention.

3. We have demonstrated beneficial *in vivo* activity in two mouse models in our laboratory that indicate that the *in vitro* results are indicative of *in vivo* utility for the claimed methods.

4. The antisense oligonucleotides used in the animal studies were prepared according to the specification of the patent application. P-ethoxy oligonucleotides and neutral phospholipids were dissolved in DMSO and tert-butanol, respectively. P-ethoxy oligonucleotides were mixed with phospholipids and excess tert-butanol (≥95% by volume) was then added. The mixture was vortexed, frozen in an acetone/dry ice bath and then lyophilized overnight. The lyophilized preparation was kept at -20°C for storage. The lyophilized oligonucleotide preparations were hydrated with normal 0.9% saline at a final oligonucleotide concentration of 40 mg/ml for the animal studies.

5. In one study, 30 nude mice, aged about 5-6 weeks, were each injected intraperitoneally with three million Johnson cells. Three groups of ten mice each were used: untreated mice (group I), liposomal Bcl-2 antisense treated mice (group II), and liposomal Bcl-2 control treated mice (group III). One week after tumor implantation, groups II and III mice were administered a biweekly intravenous injection of liposomal Bcl-2 antisense oligonucleotides (II), or liposomal Bcl-2 control oligonucleotides (III) at a dose of 15 mg/kg of mouse body weight.

In this nude mice study mice were sacrificed when they reached a moribund state, defined as a tumor size exceeding 1.5 cm^3. Mice started to reach this state after about 50 days. The study was terminated at 78 days after implantation. At 77 days post-implantation, 6 mice from each of groups I and III and only 3 mice from group II had reached moribund state.

Tissues were taken from the mice and histopathological examinations were done of liver, kidney, spleen, hear, lung, stomach intestine and bone marrow samples. Lymphoma and lymphoid hyperplasia were the most common pathological observation in the mice. The next most common was tumor involvement in the spleen. There were occasional tumors in kidney, pancreas, lung, peritoneum and bone marrow. The following results were observed:

Group I: 9 of 10 mice had lesions, hyperplasia and/or tumor involvement in the organs examined. 1 of 10 had no lesions.

Group II: 6 of 9 mice had lesions, hyperplasia and/or tumor involvement in the organs examined. 3 of 9 mice had no lesions.

Group III: 7 of 8 mice had lesions, hyperplasia and/or tumor involvement in the organs examined. 1 of 8 mice had no lesions.

Group I: 7 mice had tumor involvement in the spleen.

Group II: 2 mice had tumor involvement in the spleen.

Group III: 7 mice had tumor involvement in the spleen.

6. In a second study, SCID mice aged 4-5 weeks were injected intravenously with 50 million Johnson cells. Three groups of 5 mice each were used:

Group I: untreated mice,

Group II: mice treated with 10 mg of liposomal Bcl-2 antisense oligonucleotides per kg of mouse body weight,

Group III: mice treated with 20 mg of liposomal Bcl-2 antisense oligonucleotides per kg of mouse body weight.

One week after tumor cell implantation, group II and III mice were administered biweekly intravenous injections of the antisense oligonucleotides. The study was terminated when all untreated mice (group I) had reached a moribund state (loss of body weight, loss of motor coordination, loss of ability to eat or drink). At the termination of the study, not a single mouse in the treated groups (II and III) had reached a moribund state.

7. All statements made in this Declaration of my own knowledge are true and all statements made in this Declaration on information and belief are believed to be true, and these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 U.S.C. §1001 and may jeopardize the validity of this application or any patent issuing thereon.

1-7-98

Date

Ana M. Tari

1-7-98

Date

Gabriel Lopez-Berestein